

NATURAL HYBRIDIZATION BETWEEN
LAYIA GLANDULOSA AND *L. PANICULATA*
(ASTERACEAE: MADIINAE)

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ABSTRACT

A large putative hybrid swarm between *Layia glandulosa* and *L. paniculata* was found at La Purisima Mission State Historic Park in northern Santa Barbara Co., California. *Layia glandulosa* is a self-incompatible, morphologically and ecologically polytypic, annual, diploid species ($n = 8$) that occurs in open, relatively dry habitats. By contrast, *L. paniculata* is a self-compatible, morphologically and ecologically uniform, annual, tetraploid species ($n = 16$) that occurs in shaded, relatively moist habitats. Putative hybrids occur in two contrasting areas. In one habitat, hybrids were found in an interface between coastal sage scrub community on sandy soils on a southwest-facing slope and oak woodland-valley grassland community with sandy and clay loam soils on a north-facing slope. In the other habitat, hybrids were found in open, sandy areas between large, scattered shrubs of a coastal sage scrub-dune scrub community at the top of a mesa. Morphological analyses showed the hybrids to be intermediate in most vegetative and floral characters. The hybrid haploid chromosome number ($n = 12$) is intermediate. A meiotic analysis indicates that the modal synaptic configuration is $8_{II} + 8_I$ and suggests that these two species share a common genome. The low degree of fertility (<6%) in the hybrids presumably is the result of genic and/or chromosomal differences between the parents.

Natural hybridization has been shown to occur in several genera of tarweeds (Heliantheae: Madiinae) (Clausen 1951, Venkatesh 1958, Tanowitz 1977, Carr and Kyhos 1981). Many of these hybrids are found between species of different ploidy levels (Clausen et al. 1945, Venkatesh 1958, Tanowitz 1977, Carr and Kyhos 1981), different ecological preferences (Clausen 1951, Tanowitz 1977), and between species that are strikingly different morphologically (Kobayashi 1973, Tanowitz 1977, Carr and Kyhos 1981). Although natural hybrids occur in the Madiinae, strong external and internal reproductive isolating mechanisms operate among most members of this group (Tanowitz 1980, 1985). *Layia*, in particular, displays distinct geographical distributions among species (Clausen et al. 1945, Clausen 1951). Typically, cytological differences or strong ecological preferences are evident whenever two or more species of *Layia* occur sympatrically. Therefore, it was interesting to find a large hybrid population that occurred between two species of *Layia* that differed markedly both cytologically and morphologically.

Putative hybrids between the annual species *Layia glandulosa* (Hook.) H. & A. and *L. paniculata* Keck were found during a floristic

and vegetation study of La Purisima Mission State Historic Park in northern Santa Barbara Co., California (Ferren et al. 1984). *Layia glandulosa* (sensu lato) is self-incompatible, and highly morphologically and ecologically variable; it has large, showy heads and a chromosome number of $n = 8$; and it grows in open, relatively dry habitats (Keck 1959). At La Purisima Mission, *L. glandulosa* is found in association with coastal sage scrub and valley grassland communities in light, sandy loam soils. By contrast, *L. paniculata* is self-compatible with relatively little morphological and ecological amplitude; it has small, inconspicuous heads, and a chromosome number of $n = 16$; and it grows in shaded, relatively moist habitats (Keck 1959). At La Purisima Mission, *L. paniculata* is found in association with two distinct habitats (vegetation classification according to Cheatham and Haller 1975). The first is an oak woodland community dominated by *Quercus agrifolia* Nee in which *L. paniculata* occurs as a component of the understory on sandy and clay loam soils of north-facing slopes. The second is an unusual community that is a mixture of dune scrub dominated by *Lupinus arboreus* Sims and *L. chamissonis* Eschs. in association with typical coastal sage scrub dominated by *Baccharis pilularis* DC. subsp. *consanguinea* (DC.) C. B. Wolf in which *L. paniculata* occurs at the bases of shrubs on sandy soils on the top of a mesa. In the first habitat, hybrids occur where the coastal sage scrub community borders the oak woodland–valley grassland community where slopes were slumped and eroded. In the second habitat, hybrids occur in open areas among dominant shrubs. The latter area shows minimal natural disturbance, but significant man-made disturbance, and was the site where most of this investigation was done. Through the use of morphological, ecological, and cytological analyses, it is our purpose to document hybridization that occurs between *L. glandulosa* and *L. paniculata* and to discuss the relationships between them.

MATERIALS AND METHODS

Vegetative and floral material was prepared for observation with a dissecting scope and SEM by rehydration according to the method of Hardin (1981). For observation with the SEM, the material was critical-point dried, mounted, and sputter coated using standard procedures. Samples were examined with an Hitachi-S 415A SEM at an accelerating voltage of 25 kV.

Cytological material was examined and pollen fertility was estimated according to the methods found in Tanowitz (1985). Voucher specimens are deposited at UCSB.

RESULTS

Morphological features are shown in Table 1. Several distinctive features of *L. glandulosa* and *L. paniculata* distinguish the hybrids

TABLE 1. SALIENT MORPHOLOGICAL FEATURES EMPHASIZING THE DIFFERENCES AMONG *Layia glandulosa*, *L. paniculata*, AND THEIR HYBRIDS. Mean \pm standard deviation (and range) of measurements of parents and hybrids are from 50 individuals with one measurement for each character per individual. All length and width measurements in mm.

| Trait | <i>L. glandulosa</i> | Hybrids | <i>L. paniculata</i> |
|--|------------------------------|---------------------------------|---|
| Stem color | Anthocyanous; not dotted | Anthocyanous; dots scattered | Anthocyanous be- low; dotted throughout |
| Stem pubescence | Puberulent | Hispidulous-his- pid | Hispid |
| Lower leaf shape | Deeply pinnati- fid | Pinnatifid-den- tate | Dentate |
| Cauline leaf margin | Entire | Subentire-den- ticate | Denticulate |
| Involucre length | 7.7 \pm 1.2 (5.5-14.0) | 6.1 \pm 0.6 (5.0-7.5) | 4.9 \pm 0.5 (3.5-6.0) |
| Involucre width | 11.2 \pm 1.5 (7.0-14.5) | 8.6 \pm 1.7 (4.0-12.0) | 8.0 \pm 1.2 (5.5-11.0) |
| Receptacular bract shape | Lanceolate | Lance-linear | Linear |
| Number ray flowers/ head | 9.0 \pm 2.0 (5-13) | 9.4 \pm 2.0 (5-13) | 8.5 \pm 1.4 (6-13) |
| Ray corolla length | 16.2 \pm 3.2 (9.0-21.5) | 7.0 \pm 1.1 (4.5-9.0) | 3.9 \pm 0.6 (2.5-6.0) |
| Ray corolla width | 8.1 \pm 2.0 (3.5-15.0) | 3.3 \pm 0.6 (1.5-4.5) | 1.8 \pm 0.3 (1.5-3.0) |
| Ray corolla color | White | Cream | Yellow |
| Ray achene length | 3.4 \pm 0.6 (2.5-6.0) | 3.2 \pm 0.5 (1.5-4.5) | 2.8 \pm 0.5 (1.0-4.0) |
| Number disc flowers/ head | 64.5 \pm 12.6 (33-98) | 45.7 \pm 10.9 (17-70) | 40.0 \pm 8.6 (21-58) |
| Anther color | Yellow | Red-Brown | Black |
| Number disc flowers/ number ray flowers | 7.4 \pm 1.8 (4.4-12.8) | 4.9 \pm 0.9 (2.6-7.3) | 4.7 \pm 0.8 (3.4-7.7) |
| Disc corolla length | 4.8 \pm 0.7 (2.5-6.0) | 3.8 \pm 0.5 (3.0-5.0) | 3.1 \pm 0.4 (2.5-4.0) |
| Disc achene length | 3.6 \pm 0.7 (1.5-5.0) | 3.3 \pm 0.7 (1.5-4.5) | 3.2 \pm 0.4 (2.5-4.5) |
| Pappus color | White | Light Brown- White | Rufous |
| Ray corolla length/ disc corolla length | 3.4 \pm 0.7 (1.7-5.0) | 1.8 \pm 0.2 (1.2-2.7) | 1.3 \pm 0.2 (0.8-1.6) |

from these species in the field. For example, *L. glandulosa* has large, showy heads with broad, trilobate, white rays, whereas *L. paniculata* has smaller heads with inconspicuous, strap-shaped, yellow rays. Putative hybrids typically displayed intermediate-sized heads with conspicuous cream-colored rays. Hybrids showed some variation in ray corolla color, but no hybrids were observed to possess either all white or all yellow corollas. Qualitative characters (e.g., stem color,

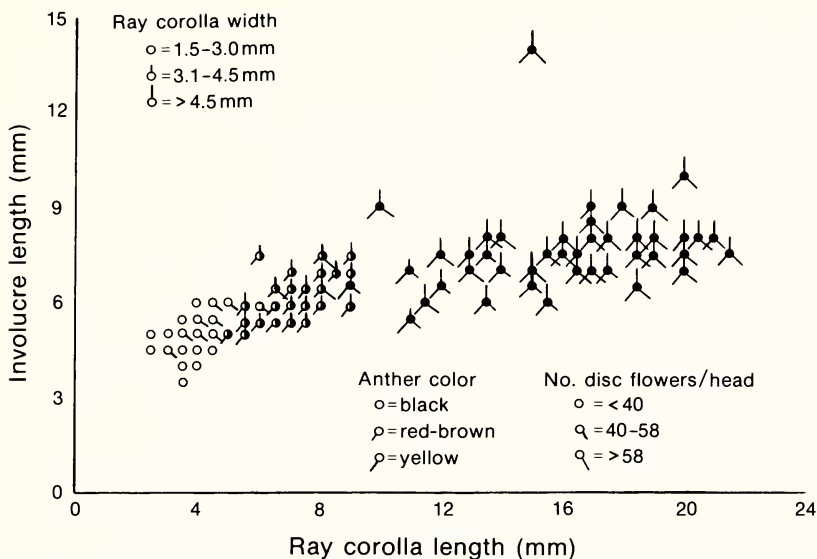


FIG. 1. Scatter diagram that depicts 50 individuals each of *Layia glandulosa*, *L. paniculata*, and their hybrids from the study site. Closed circles represent white ray corollas of *L. glandulosa*, open circles represent yellow ray corollas of *L. paniculata*, and half circles represent cream-colored ray corollas of hybrids. Several individuals with a similar morphology overlap and are not indicated.

branching) showed similar patterns of variation that suggest simple dominance is lacking in these features. In most other instances, hybrids were intermediate in vegetative and floral characters. Both parental species have a campanulate involucre, but *L. glandulosa* has larger heads with greater numbers of ray and disc flowers per head.

An SEM analysis of the vesture of the stem, lower and cauline leaves, and involucre revealed distinct differences in types and density of trichomes between the parental species. Hybrids typically showed intermediacy in density and possessed combinations of both parental trichome types on all structures examined. For example, *L. paniculata* possesses sessile, biseriate, glandular trichomes on the involucre, which are lacking in *L. glandulosa* (cf. Carlquist 1958, Figs. 3, 8). These trichomes were found on the involucre in all hybrid individuals, but had a more scattered distribution. The setose pappus elements are barbellate in both species, but the shorter outer elements of *L. paniculata* were found to be tightly spiralled and hygroscopic in action. Spiralled pappus elements were lacking in *L. glandulosa*, but occurred in some hybrids.

Morphological data for individuals are depicted in a scatter diagram (Fig. 1). The shape of the recombination spindle suggests that

the putative hybrids are F_1 's. The majority of hybrids exhibited character states intermediate to one or the other parental species. No evidence of introgression or backcrossing was present. The scatter diagram revealed further that individuals of *L. paniculata* are clustered tightly, which suggests that this population is relatively uniform morphologically. This is in contrast to individuals of *L. glandulosa* that display scattered points, which presumably reflect the greater morphological amplitude in this population. Although a few individuals of *L. paniculata* had numerous disc flowers ($n > 58$), most individuals of *L. glandulosa* had disc flowers well in excess of those of *L. paniculata*. Moreover, those *L. glandulosa* individuals with fewer disc flowers also were those that possessed smaller involucre and shorter ray corollas. Only three hybrid individuals showed numbers of disc flowers greater than 58, and none had ray corollas longer than 9 mm.

An analysis of meiotic features was performed using pollen mother cells (PMC's) obtained from buds of the parents and putative hybrids. *Layia glandulosa* (12 individuals, 84 PMC's) showed a chromosome number of $n = 8$, whereas *L. paniculata* (15 individuals, 67 PMC's) showed a chromosome number of $n = 16$. Meiosis in both species appeared normal in all instances and showed no apparent structural heterozygosity in the population. Putative hybrids (10 individuals, 127 PMC's) had a chromosome number of $2n = 24$. The modal synaptic configuration at diakinesis or metaphase I was $8_{II} + 8_I$. Six PMC's showed $1_{III} + 6_{II} + 9_I$, with the association of three as a chain. One PMC showed $1_{IVch} + 6_{II} + 8_I$, whereas four PMC's had fewer bivalents and more univalents (maximum of 12). Anaphase I and II cells showed no evidence of any major structural chromosomal rearrangements (e.g., bridges or fragments); however, unpaired chromosomes often were observed lagging on the equator. In three PMC's, two to three bivalents appeared to undergo asynchronous disjunction. Telophase II cells showed micronuclei.

Fifty individuals each of *L. glandulosa* and *L. paniculata*, used in morphological analyses, were examined for pollen fertility (500 grains/individual). Pollen stainability in both species averaged above 98% and ranged 87–100% in *L. glandulosa*, and 81–100% in *L. paniculata*. Similarly, 50 hybrid individuals were examined for pollen fertility. Pollen stainability averaged nearly 1% and ranged 0–6% (500 grains/individual).

Over 2000 fruits were visually examined in each species and over 6000 among all hybrids. Fruit set was 73% in *L. glandulosa*, 77% in *L. paniculata*, and 0% in the hybrids.

DISCUSSION

Natural hybridization between *L. glandulosa* and *L. paniculata* is apparent from the morphological data. The large morphological dif-

ferences evident between the parental species were found to be intermediate in the hybrid population in most features. Cytological data showed a triploid condition in hybrids and so provide additional evidence for hybridity. Furthermore, we consider all hybrid individuals studied in the population to be F_1 's, based on extremely high pollen sterility, meiotic anomalies, and lack of fruit set.

In spite of striking, obvious, morphological differences, *L. glandulosa* and *L. paniculata* share less obvious features (e.g., involucre shape, pappus morphology, and vesture). These characters, although found in other members of the genus, are confined principally to what comprises a morphologically and cytologically closely related phyletic group (Clausen 1951, Tanowitz unpublished). These phenotypic similarities presumably reflect genic and chromosomal similarities. Cytogenetic analyses suggest that the two species share a common genome. Additional meiotic analyses of artificial hybrids, in progress, should reveal the extent of homology that occurs between *L. glandulosa* and *L. paniculata*.

Layia glandulosa and *L. paniculata* are ecologically distinct throughout most of their respective ranges. Even in areas of close sympatry these species typically retain strong microhabitat preferences. Several distinct populations of *L. glandulosa* and *L. paniculata* occur at La Purisima Mission (Ferren et al. 1984). In only one area of high disturbance do the ecological barriers break down sufficiently to provide an intermediate habitat to support both parents and their hybrids. The other habitat in which hybrids occur is unusual. This habitat represents a unique association and provides a situation that also results in the breakdown of ecological barriers. Because the morphological and cytological differences between the parents do not preclude crossability, it appears then that the reproductive isolating mechanisms are fundamentally external. Following the breakdown of external barriers, internal mechanisms, such as hybrid sterility, presumably reinforce their distinctness in contiguous populations.

Although natural hybridization in Madiinae has been well-documented (Clausen et al. 1945, Clausen 1951, Carr and Kyhos 1981, Tanowitz 1977, 1980), studies of artificial hybridization in the subtribe suggest that the potential for hybridization is even greater than observed in nature (Clausen 1951, Carr 1975, 1977, 1978, Tanowitz 1980). Ecological and distributional factors operate as strong reproductive isolating mechanisms in the Madiinae. Clausen (1951) noted that many genera, including *Layia*, display strong ecological preferences and distributional disjunctions. Clausen (1951) further noted that where *Layia* species occur sympatrically they typically comprise two fundamentally different cytological groups (species complexes) that display strong barriers against interbreeding. *Layia glandulosa* and *L. paniculata* are in the same phyletic group, but are considered to be in a different morphological complex from their common

cytological group (Clausen 1951). *Layia glandulosa* has the greatest eco-distributional amplitude of any taxon in the subtribe (Clausen et al. 1945). The distribution of *L. glandulosa* completely encompasses that of *L. paniculata*, yet they are distinct ecologically. Thus, the lack of natural hybridization in these two species is not unexpected.

Hoover (1970) reported a natural hybrid between *L. glandulosa* and *L. hieracioides* (DC.) H. & A. (Hoover 8369 OBI!) in San Luis Obispo Co. The parentage of this hybrid is problematical. The diploid *L. hieracioides* is one of the presumed progenitors of the tetraploid *L. paniculata*. Preliminary investigations of these two species indicate that their distributions overlap, ecological preferences are similar, and morphological characters used in their discrimination segregate randomly among populations (Tanowitz, unpublished). Hoover (1970) reported no cytological information and so it is difficult to decide whether Hoover 8369 is a cross of *L. glandulosa* with *L. hieracioides* or with *L. paniculata*.

The only other report of natural hybridization in *Layia* was reported by Clausen et al. (1945). They discovered a putative hybrid individual between *L. pentachaeta* A. Gray ($n = 8$) and *L. platyglossa* (Fisch. Meyer) A. Gray ($n = 7$) in southeastern Monterey Co., California. This individual showed an intermediate morphology, a chromosome number of $2n = 15$, and was highly sterile. It also shared morphological and cytological similarities with an artificially produced hybrid of the same combination (Clausen et al. 1945). They showed that through spontaneous chromosome doubling, the artificial hybrid gave rise to a tetraploid individual, and designated it as '*L. pentaglossa*'. They suggested that the presence of unreduced gametes found in the artificial hybrid increased the possibility that the tetraploid could occur in nature. This possibility appears remote, however, because '*pentaglossa*' was derived from obligate outcrossing species and, thus, would have to pass through a bottleneck. A similar origin for the self-fertile, tetraploid *L. paniculata* is more likely because it was apparently derived, in part, from a self-fertile species, *L. hieracioides*.

It has been suggested that a relatively common mechanism for speciation in the Madiinae is through hybridization and subsequent chromosomal repatterning that may or may not result in aneuploidy (Venkatesh 1958, Carr 1975, 1977, Tanowitz 1977, 1985). Speciation occurring without change in chromosome number has been documented in a number of examples in this group, although several genera show aneuploid series (Carr 1975, 1977, Tanowitz 1980, Carr and Kyhos 1981). In contrast, speciation as the result of hybridization and subsequent polyploidy in the Madiinae is apparently uncommon. Along with genetic and ecologic isolation factors, this mode of speciation is a result presumably of the bottleneck effect

through which species must pass (Stebbins 1950). Clausen et al. (1945), however, demonstrated that the hexaploid *Madia citrigracilis* Keck ($n = 24$) resulted from a cross of *M. gracilis* (Sm.) Keck ($n = 16$) with *M. citriodora* Greene ($n = 8$). It appears that most, if not all, polyploid species in this group are self-fertile and have been derived from at least one parent that is self-fertile. Therefore, the possibility exists that hybridization and subsequent polyploidy could serve as a mechanism for speciation among self-fertile species in the Madiinae.

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